| | Type | L # | Hits | Search Text | DBs | Time Stamp |
|---|---|---|---|---|---|---|
| 1 | BRS | L1 | 0 | (dielectric adj matric) same (nano adj3 magnetic adj3 particles) | USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB | 2002/10/17 16:53 |
| 2 | BRS | L2 | 3 | (dielectric adj matrix) same (nano adj3 magnetic adj3 particles) | USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB | 2002/10/17 16:56 |
| 3 | IS&R | L3 | 147 | (257/642).CCLS. | USPAT | 2002/10/17 16:57 |
| 4 | BRS | L4 | 1 | 3 and (dielectric adj3 matrix) | USPAT | 2002/10/17 17:08 |
| 5 | BRS | L5 | 17976 | matrix same (silica or alumina or hydrosilsesquioxane or polymide or PMMA or methylsilsesquioxane) | USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB | 2002/10/17 17:25 |
| 6 | BRS | L6 | 228 | 5 same magnetic | USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB | 2002/10/17 17:28 |

| | Type | L # | Hits | Search Text | DBs | Time Stamp |
|---|---|---|---|---|---|---|
| 7 | BRS | L7 | 21 | 6 and (superparamagnetic) | USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB | 2002/10/17 17:28 |

US-PAT-NO: 6368800

DOCUMENT-IDENTIFIER: US 6368800 B1

TITLE: Kits for isolating biological target materials using silica magnetic particles

---------- KWIC ---------

The magnetic particles designed to bind nucleic acid materials indirectly are generally used to isolate a specific nucleic acid material, such as mRNA, according to the following basic isolation procedure. First, a medium containing a nucleic acid material is placed in contact with a label capable of binding to the nucleic acid material of interest. For example, one such commonly employed label, biotinylated oligonucleotide deoxythymidine (oligo-dT), forms hydrogen bonds with the poly-adenosine tails of mRNA molecules in a medium. Each label so employed is designed to bind with a magnetically responsive particle, when placed into contact with the particle under the proper binding conditions. For example, the biotin end of a biotinylated oligo-dT/mRNA complex is capable of binding to streptavidin moieties on the surface of a streptavidin coated magnetically responsive particle. Several different commercial sources are available for streptavidin magnetic particles and reagents designed to be used in mRNA isolation using biotinylated oligo-dT as described above. See, e.g. PolyATtract.RTM. Series 9600.TM. mRNA Isolation System from Promega Corporation; or the ProActive.TM. line of streptavidin coated microsphere particles from Bangs Laboratories

(Carmel, Ind., U.S.A.). Magnetic particles and label systems have also been developed which are capable of indirectly binding and isolating other types of nucleic acids, such as double-stranded and single-stranded PCR templates. See, e.g. BioMag.TM. **superparamagnetic** particles from Advanced Magnetics, Inc. (Cambridge, Mass., U.S.A.)

As used herein, the term "magnetic particles" refers to materials which have no magnetic field but which form a magnetic dipole when exposed to a magnetic field, i.e., materials capable of being magnetized in the presence of a magnetic field but which are not themselves magnetic in the absence of such a field. The term "magnetic" as used in this context includes materials which are paramagnetic or **superparamagnetic** materials. The term "magnetic", as used herein, also encompasses temporarily magnetic materials, such as ferromagnetic or ferrimagnetic materials with low Curie temperatures, provided that such temporarily magnetic materials are paramagnetic in the temperature range at which silica magnetic particles containing such materials are used according to the present methods to isolate biological materials.

The term "**silica magnetic** particle" refers to a **magnetic** particle comprised of **silica** in the form of **silica** gel, siliceous oxide, solid **silica** such as glass or diatamaceous earth, or a mixture of two or more of the above. The term "**silica** gel" as used herein refers to chromatography grade **silica** gel, a substance which is commercially available from a number of different sources. **Silica** gel is most commonly prepared by acidifying a solution containing silicate, e.g. sodium silicate, to a pH of less than 10 or 11 and then allowing the acidified solution to gel. See, e.g. **silica**

preparation discussion in Kurt-Othmer Encyclopedia of Chemical Technology, Vol. 6, 4th ed., Mary Howe-Grant, ed., John Wiley & Sons, pub., 1993, pp. 773-775. The term "**silica magnetic** particle" as used herein preferably refers to particles with the characteristics described above having the capacity to bind at least 2 micrograms of biological target material per milligram of **silica magnetic** particles and, independently, the capacity to release at least 60% of the biological target material bound thereto in the elution step of the present method. The **silica magnetic** particles used in the present invention preferably further comprise ferromagnetic material incorporated into a **silica** gel **matrix**.
The elution step in the isolation methods of this invention are preferably accomplished without substantial contamination of the nucleic acid material by metal or metal compounds (e.g., iron or iron compounds) or other objectionable species originating from the **silica magnetic** particles.

The term "siliceous-oxide coated magnetic particle" or "SOCM particle" is used herein to refer to the most preferred form of silica magnetic particle used in the methods and kits of the present invention. The SOCM particle is comprised of siliceous oxide coating a core of at least one particle of **superparamagnetic** or paramagnetic material. The SOCM particle used in the present method and kits also has an adsorptive surface of hydrous siliceous oxide, a surface characterized by having silanol groups thereon. Target nucleic acid material, such as DNA or RNA, adhere to the adsorptive surface of the particle while other material, particularly deleterious contaminants such as exonucleases, do not adhere to or co-elute from the particle with the nucleic acid materials.
The physical characteristics of the SOCM particle and

methods for producing
such particles are disclosed in concurrently filed U.S.
patent application
Ser. No. 08/786,600, entitled "Silica Adsorbent on
Magnetic Substrate," the
disclosure of which is incorporated by reference herein.

Preparation of **Superparamagnetic** Iron Oxide Particles

Hydrogen Peroxide Treatment of **Superparamagnetic** Particles

US-PAT-NO: 5945525

DOCUMENT-IDENTIFIER: US 5945525 A

TITLE: Method for isolating nucleic acids using silica-coated magnetic particles

---------- KWIC ---------

A nucleic acid-bondable magnetic carrier of the present invention is a magnetic silica particle comprising a **superparamagnetic** metal oxide, wherein the magnetic silica particle has a specific surface of about 100 to about 800 m.sup.2 /g.

In conventional methods for isolating a nucleic acid using a nucleic acid-bondable magnetic carrier, it is known to utilize a magnetic-responsible particle having a **superparamagnetic** iron oxide core covered with a polymeric silane layer to which a biocompatible molecule (for example, a nucleic acid) is covalently bonded (Japanese Laid-Open Patent Publication No. 60-1564).

A method for determining a ligate concentration including the following steps is also known: (1) using a magnetic-responsible particle containing **superparamagnetic** iron oxide covered with a polymeric silane layer to which a biocompatible molecule is capable of being bonded; (2) reacting a sample solution containing a ligate, a known amount of a labeled ligate, and the magnetic-responsible particle to which a ligate-specific ligand is bonded, so as to form a ligand-ligate complex on the magnetic-responsible particle; (3) magnetically separating the magnetic-responsible particle

from the reaction solution; (4) measuring the labeled ligate which is bonded to the magnetic-responsible particle or free labeled ligate in the reaction solution; and (5) applying the measurement of the label ligate to the standard curve so as to obtain the ligate concentration. This method is described in Japanese Patent Publication No. 7-6986.

A **superparamagnetic** particle with a plurality of separated oligonucleotide sequences having monodispersibility (less than 5% of particle diameter distribution), and a method for producing a magnetic particle which covalently bonds or adsorbs the oligonucleotides to functional groups (for example, biotinyl groups) or molecules on the surface thereof is known. It is also known to utilize a particle to which oligonucleotide is covalently bonded or adsorbed as a probe of a nucleic acid (WO90/06045).

The nucleic acid-bondable magnetic carrier of the present invention is a magnetic silica particle containing a **superparamagnetic** metal oxide, wherein the magnetic silica particle has a specific surface of about 100 to about 800 m.sup.2 /g.

In one embodiment of the present invention, the **magnetic silica** particle is a composite of a **superparamagnetic** metal oxide having a surface covered with **silica** and an inorganic porous **matrix** material composed of fine **silica** particles, and is substantially spherical.

In another embodiment of the present invention, the **superparamagnetic** metal oxide is iron oxide.

In still another embodiment of the present invention, the **superparamagnetic**

metal oxide is contained in an amount of about 10 to about 60 percent by
weight.

mixing a nucleic acid-bondable magnetic carrier which is a magnetic silica
particle containing a **superparamagnetic** metal oxide and has a specific surface
of about 100 to about 800 m.sup.2 /g, a material containing a nucleic acid and
a solution for extracting the nucleic acid so as to form a sample solution;

mixing a nucleic acid-bondable magnetic carrier which is a magnetic silica
particle containing a **superparamagnetic** metal oxide crystal and has a specific
surface of about 100 to about 800 m.sup.2 /g, a material containing a nucleic
acid and a solution for extracting the nucleic acid so as to form a sample
solution;

According to still another aspect of the invention, a kit for isolating a
nucleic acid includes a nucleic acid-bondable magnetic carrier which is a
magnetic silica particle containing a **superparamagnetic** metal oxide and has a
specific surface of about 100 to about 800 m.sup.2 /g, and a solution for
extracting the nucleic acid.

A nucleic acid-bondable magnetic carrier of the present invention is a magnetic
silica particle containing a **superparamagnetic** metal oxide. The magnetic
silica particle of the present invention is capable of bonding a nucleic acid
and separating solid and liquid by utilizing a magnetic field.

In a preferred embodiment of the invention, the **magnetic silica** particle of the
present invention is a composite of the **superparamagnetic** metal oxide having a
surface covered with **silica** and an inorganic porous **matrix** material composed of

fine **silica** particles. The **magnetic silica** particle is substantially spherical.

The **superparamagnetic** metal oxide used in the present invention refers to the metal oxide which is responsive to a magnetic field variation but is not permanently magnetized, and has a small residual magnetization.

A preferred example of the **superparamagnetic** metal oxide is iron oxide. As iron oxide, triiron tetraoxide (Fe.sub.3 O.sub.4), iron sesquioxide (.gamma.Fe.sub.2 O.sub.3), which is obtained by gradually oxidizing triiron tetraoxide, and the like may be used. Triiron tetraoxide is especially preferably used. The **superparamagnetic** metal oxide is preferably in the form of particle, and more preferably in the form of a substantially spherical particle. The diameter of the **superparamagnetic** metal oxide is preferably in the range of about 0.2 to about 0.4 .mu.m, more preferably in the range of about 0.25 to about 0.30 .mu.m. Since triiron tetraoxide having a substantially spherical form has an especially small residual magnetization and a smooth surface, it can be used repeatedly in separating operations. Furthermore, the magnetic silica particle containing triiron tetraoxide has excellent stability in neutral and weak acidic aqueous solutions and is capable of being stored more than two years in the solution.

The amount of the **superparamagnetic** metal oxide contained in the magnetic silica particle of the present invention may vary depending on the magnetization intensity of the metal oxide; however, the amount is preferably in the range of about 10 to about 60 percent by weight, more preferably in the range of about 20 to about 40 percent by weight. By

providing the
superparamagnetic metal oxide in the magnetic silica particle in such a
preferable range, the magnetic carrier (i.e., the magnetic silica particle) can
be rapidly separated from the sample solution utilizing commercially available
magnets.

The most preferred magnetic silica particle satisfies the following
requirements: (1) it contains a superparamagnetic iron oxide; (2) it has a
specific surface of about 100 to about 800 m.sup.2 /g; (3) the iron oxide is
almost covered with silica; (4) it is a composite of the iron oxide covered
with silica and an inorganic porous matrix material composed of fine silica
particles; (5) it contains the iron oxide in an amount of about 10 to about 60
percent by weight; (6) it has an average surface pore diameter of about 0.1 to
about 60 nm; (7) it has a pore volume of about 0.01 to about 1.5 ml/g; and (8)
it has a particle diameter of about 0.5 to about 15 .mu.m.

A method for isolating a nucleic acid of the present invention includes the
steps of mixing a nucleic acid-bondable magnetic carrier, which is a magnetic
silica particle containing a superparamagnetic metal oxide and has a specific
surface of about 100 to about 800 m.sup.2 /g, a material containing a nucleic
acid and a solution for extracting the nucleic acid so as to form a sample
solution; separating the magnetic carrier to which the nucleic acid has been
bonded from the sample solution using a magnetic field; and eluting the nucleic
acid from the magnetic carrier to which the nucleic acid has been bonded.

A kit for isolating a nucleic acid of the present invention comprises a nucleic
acid-bondable magnetic carrier which is a magnetic silica particle containing a

**superparamagnetic** metal oxide and has a specific surface of about 100 to about
800 m.sup.2 /g, and a solution for extracting the nucleic acid.

(1) mixing a polynucleotide-bondable magnetic particle with a solution
containing a polynucleotide and a solution of a chaotropic agent, wherein said
magnetic particle is a composite of a **superparamagnetic** metal oxide and a fine
silica and wherein the specific surface of said magnetic particle is about 100
to about 800 square meters per gram;

3. A method for isolating a polynucleotide according to claim 1, wherein the
**superparamagnetic** metal oxide is iron oxide.

4. The method for isolating a polynucleotide according to claim 1, wherein the
magnetic particle contains from about 10 to about 60 percent by weight of the
**superparamagnetic** metal oxide.

12. A kit for isolating a polynucleotide comprising a magnetic silica particle
and a chaotropic agent, wherein said magnetic silica particle is a composite of
a **superparamagnetic** metal oxide and fine silica and wherein said magnetic
silica particle possesses a specific surface of about 100 to about 800 square
meters per gram.